A Technique to Expose Animals to Concentrated Fine Ambient Aerosols

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This paper presents the development and evaluation of an ambient particle concentrator for conducting animal inhalation exposure studies. The system utilizes the principle of virtual impactors to concentrate ambient particles in the size range 0.1–2.5 µm (aerodynamic diameter; d_p) by drawing them through a series of three virtual impactors. Each impactor contains the majority of ambient fine mass ($d_p < 2.5$ µm aerodynamic diameter) in a bleed flow (minor flow) that is 20% of the total flow entering the virtual impactor. The virtual impactors have been characterized using indoor air samples as test aerosols. Fine mass and sulfate concentrations at the outlet of the concentrating system were compared to the ambient fine mass and sulfate levels, which were determined using Harvard-Marple impactors. In each of the stages, particle concentration was increased by a factor of approximately 3. Thus, an overall concentration factor of about 25–30 was achieved. The main goal of this study was to demonstrate the feasibility of conducting animal exposures using the newly developed ambient fine particle concentrator. *Key words*: ambient particles, animal inhalation exposures, concentrator, virtual impactors. *Environ Health Perspect* 103:172–177 (1995)

Ambient particles consist both of natural materials such as pollen and anthropogenic materials such as acid aerosols. They can be directly emitted by different sources (primary aerosols) or can be formed during gas-to-particle conversion processes (secondary aerosols). Both the concentration and the size of the particles depend on a number of factors including the nature of the emissions, photochemical activity, and meteorological events. Particles are usually classified into two categories: coarse [particle aerodynamic diameter (d_p) >2.5 µm] and fine ($d_p \leq 2.5$ µm). The first category generally includes particles produced through mechanical processes acting on such materials as soil or water, while the second category includes particles formed through gas-to-particle conversion, as in the case of acid sulfates or products of incomplete combustion such as soot.

The major chemical components of inhalable particles are sulfate, nitrate, and ammonium ions, organic and elemental carbon, as well as a variety of trace metals formed in combustion processes. Oxidation of the primary gaseous pollutants sulfur dioxide and nitrogen oxides, emitted during the combustion of coal, oil, and other conventional fuels, results in the formation of sulfuric and nitric acid that occurs through homogeneous or heterogeneous atmospheric processes. Ammonium is formed through the neutralization of these acids by ambient ammonia. There are a variety of potential sources of trace metals found in the inhalable ambient particulate matter, including coal and oil combustion, wood burning, waste incineration, and metal mining and production. In addition to sulfate, nitrate, and metals, carbon-containing compounds are also associated with inhalable particles. A variety of organic compounds, including long-chain hydrocarbons, polycyclic aromatic hydrocarbons, and organics containing oxygen, nitrogen, or sulfur, can be present at nanogram per cubic meter to microgram per cubic meter levels. In addition to the semi- and nonvolatile organics, a host of viable particulate species can be present such as fungi, bacteria, pollen, yeasts, and viruses.

Because fine particles are capable of penetrating deeply into the respiratory system, most of the health studies have focused on the inhalable portion of the particle size spectrum. Twenty years ago, the U.S. EPA included total suspended particles (TSP) as one of eight criteria pollutants. Recently, TSP has been replaced by PM_{10} (particles with an aerodynamic diameter of less than 10 µm) because PM_{10} is a measurement more relevant to respiratory health. Considering that the chemical composition and toxicity of particles can vary with time and location, particle mass concentration alone may not be a sufficient criterion for protecting the public health. For instance, exposure to an equal amount and particle size of soil dust and acid aerosol would not necessarily result in similar adverse respiratory health effects.

A number of epidemiological studies have underlined the association between mortality and morbidity effects and high ambient particle concentration levels. These studies have been reviewed by Ware et al. (*1*) and more recently by Ozkaynak (*2*). Laboratory animal inhalation studies using artificial preparations have demonstrated moderate or no effects at concentrations much higher than those typically found in the ambient air (*3,4*). These studies have relied on controlled-chamber exposures to single chemical component particles or a simple mixture of a few components. Such artificial particles are not an accurate reproduction of ambient respirable particles, which typically consist of a mixture of polycyclic aromatic hydrocarbons, heavy metals, quartz, sulfate, and adsorbed semivolatile compounds (internally or externally mixed). These components in the ambient particles, most likely, interact together in creating toxic effects (*5*).

To assess adequately the toxicity of ambient aerosols, we need to develop a clear understanding of the biological effects of the inhaled material. To date, it has not been possible or practical to perform controlled exposures to ambient particles under robust conditions that are useful for environmental toxicology and risk assessment (*6*). This is due to the fact that ambient levels are usually not high enough to perform dose-response assessments. The purpose of this study is to present a new method that enables us to study ambient particle toxicity and pulmonary effects in a real-time, dynamic inhalation system. The key feature of this method is the use of a series of virtual impactors to concentrate ambient particles without altering their size distribution (due to coagulation or excessive losses) and physicochemical characteristics.

Methods

Main components of the concentrating system. The virtual impactor concept can be used to construct an aerosol concentrator for a desired particle size range. The virtual impactor (Fig. 1) is a device used to classify particles according to their aerodynamic size. In this device, a jet of particle-laden air is injected at a collection probe, which is slightly larger than the acceleration nozzle. Particles larger than a certain size cross the air streamlines and enter the collection probe, while particles smaller than a certain size follow the deflected streamlines. To remove the larger particles from the injected air, a fraction of the total flow is allowed to pass through the probe; this is

referred to as the minor flow (typically 10–20% of the total flow). As a result, the concentration of the larger particles in the minor flow is increased by a factor of Q_{tot}/Q_{min}, where Q_{tot} is the total flow entering the virtual impactor and Q_{min} is the minor flow. Because the mass fraction of ambient particles smaller than approximately 0.1 μm aerodynamic diameter is negligible (7–9), the minor flow of a virtual impactor with a 50% cutpoint on the order of 0.1 μm contains essentially all the fine ambient particulate mass.

The ambient particle concentrator (Fig. 2) used for animal inhalation studies consists of the following components: 1) a high-volume conventional impactor with a 2.5 μm cut-off size (separator); 2) a series of three virtual impactors with a 0.1 μm cut-off size (concentrators I, II, and III); 3) a series of honeycomb denuders that remove gaseous pollutants from the concentrated aerosol to be supplied to the exposure chamber; and 4) an animal exposure chamber. A detailed description of each of the components of the system is presented below.

Conventional impactor (separator). The first impactor is a high-volume conventional impactor (fractionating sampler; Andersen Samplers, Inc., Atlanta, Georgia) and removes particles larger than 2.5 μm operating at 1000 l/min, while particles smaller than 2.5 μm escape collection. The impactor has been characterized in detail elsewhere (10). A preselective inlet that removes particles larger than 10 μm in diameter can be used instead of the $PM_{2.5}$ inlet to concentrate inhalable (d_p<10 μm) instead of fine particles, if desired.

Virtual impactors. The deflected flow of the conventional impactor, which contains particles smaller than 2.5 μm in aerodynamic diameter (1000 l/min), is drawn through a series of three virtual impactors (Fig. 2). The concentration of particles smaller than 2.5 μm is increased using each virtual impactor with a cutpoint at 0.1 μm. The minor flow of the virtual impactor contains the concentrated aerosol. The design of the concentrator is based on a previously developed prototype for human inhalation studies (11). In that device, ambient aerosols were concentrated as they passed through two virtual impactors in series, each one containing most of the ambient fine mass (d_p<2.5 μm aerodynamic diameter) in a bleed (minor) flow that is 20% of the total flow entering the virtual impactor. The output flow rate of the concentrated aerosol was 50 l/min. In each stage, the concentration is increased by a factor of about 3. Because concentrated ambient aerosols for animal exposure studies need to be supplied to the exposure chamber at substantially smaller flow rates
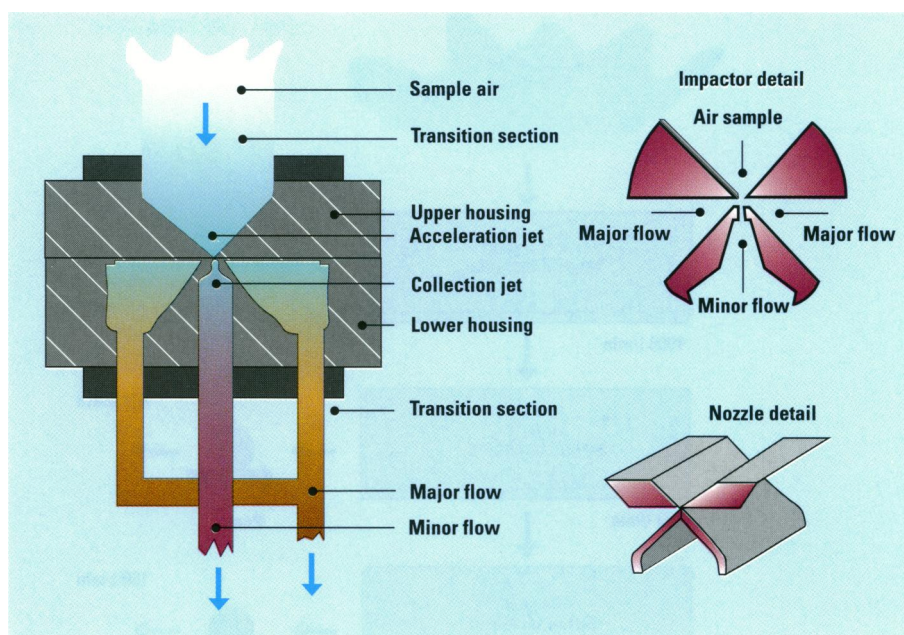

Figure 1. Schematic diagram of a virtual impactor.

(10 l/min or less), a third virtual impactor was placed downstream of the original two-stage concentrator. The total flow rate into the third stage is 50 l/min and concentrated particles are supplied to the exposure chamber at 10 l/min. A detailed performance evaluation of this virtual impactor system has been described elsewhere (12).

Honeycomb denuders. When removal of certain gas-phase pollutants is required, the aerosol can be drawn through a series of glass honeycomb denuders that selectively remove ozone, ammonia, and sulfur dioxide at 10 l/min (Fig. 2) before administering the concentrated aerosol in the exposure chamber. Concentrations of these gases are usually correlated with particle concentrations during air pollution episodes, and their removal minimizes confounding interferences, which would make the investigation of respiratory effects of particles difficult. Furthermore, the system is designed in such a way to allow introduction of known amounts of gaseous pollutants (e.g. ozone) to the exposure chamber, along with the concentrated ambient aerosol, to study the synergistic effects of different gases.

The glass honeycomb denuders have been evaluated at a flow rate range of 1.5–10 l/min (13). Their collection efficiency for reactive gases, such as SO_2, HNO_3, and NH_3, are approximately 99%. In addition, particle losses through these denuders were found to be 5% or less (14).

Animal exposure chamber. The animal exposure chamber is a 60-l plexiglass tube (30.48 cm in diameter and 82 cm long). The concentrated aerosol is supplied to the chamber through a brass tube, 1.9 cm in

diameter and 15 cm long. The tube is connected to the outlet of the minor flow of stage III. Since the aerosol is supplied at 10 l/min, the mean residence time in the chamber is 6 min. The relatively short residence time minimizes particle losses due to deposition on the chamber walls. The average residence time of the sampled aerosol in the concentrating system (virtual impactors and transition pieces) is on the order of few seconds. Therefore, the aerosol residence time in the entire exposure unit is about 6 min, which gives an estimate of the time-constant of the system with regard to responsiveness to changes in the ambient concentration.

A large amount of ammonia is generated in the chamber during animal exposures, which could neutralize sulfate or other acid aerosols. If it is necessary to prevent neutralization of acid aerosols, the walls of the chamber can be coated with citric acid to trap the ammonia over the exposure period. Although this chamber is designed for whole-body exposures, the outlet of the third virtual impaction stage can also be connected to a nose-only exposure chamber, if it is desirable. One of the advantages of a nose-only chamber is that acidity neutralization can be prevented without coating the walls of the chamber with citric acid.

Characterization of the concentrating system. Indoor aerosol-concentrating experiments were conducted at the Harvard School of Public Health during the winter of 1993–94. We estimated the ambient levels of fine particulate mass ($PM_{2.5}$) and sulfate using two colocated Harvard-Marple Impactors (HMIs). The
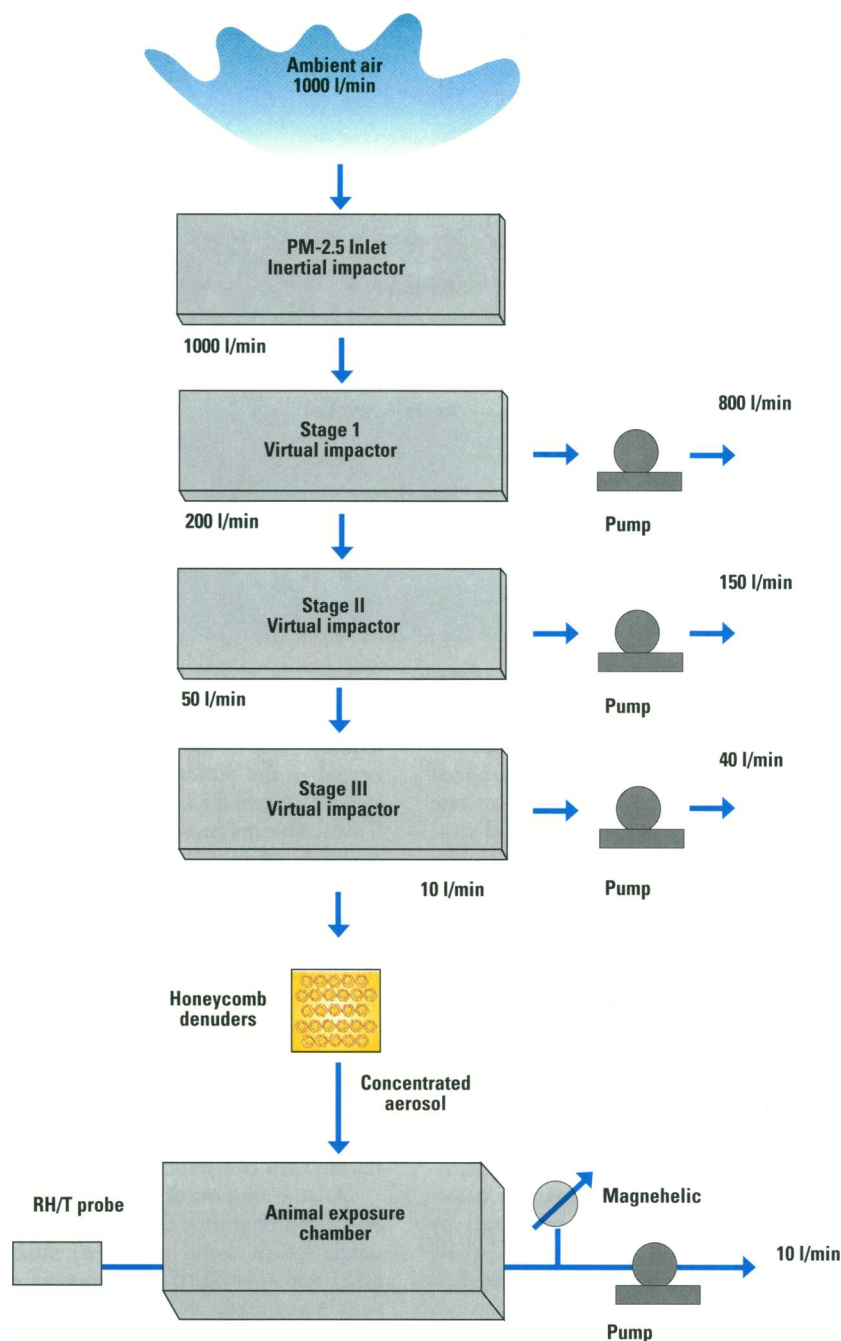
Figure 2. Schematic diagram of the concentrating system to conduct animal exposures.

Particle losses in the exposure chamber. To determine possible losses due to particle deposition on the walls of the exposure chamber, a Teflon filter was placed upstream from the chamber, immediately after the outlet of the concentrator, and another Teflon filter was placed downstream from the chamber. The upstream filter was connected to a pump sampling at 1 l/min, whereas the downstream filter was connected to a pump sampling at 10 l/min. Hence, the minor flow of the third stage was set at 11 l/min instead of 10 l/min to maintain the required flow through the chamber as well as to determine the concentration upstream in the chamber. The experiments were conducted without any animals or animal cages in the chamber. At the end of each run, the filters were extracted similarly to the process described above and analyzed for sulfate ion by ion chromatography.

Results

The first step in characterizing the concentrating system was to investigate the effect of the minor-to-total flow ratio on the performance of the virtual impactor. Lower minor-flow ratios (q_m/Q_T) theoretically result in higher concentration levels of the exposure aerosol. However, previous investigations (*12,17*) show that low minor flow ratios (10% or less) tend to increase internal particle losses, as well as the impactor's collection efficiency.

The minor flow of stage III was set at 5 and 10 l/min (the minor flow ratio, q_m/Q_T varied from 0.10 to 0.20), and the total intake sampling flow rate was maintained constant at 50 l/min. Results of this investigation are shown in Figures 3–6. Table 1 presents a summary of the experimental characterization of the concentrating system. Indoor fine mass concentrations varied from 5.5 to 31.8 $\mu g/m^3$, whereas the indoor sulfate levels varied from 1.3 to 7.3 $\mu g/m^3$. Depending on the ambient levels, fine mass concentrations as high as 700 $\mu g/m^3$ and sulfate concentrations as high as 121.5 $\mu g/m^3$ have been achieved by the concentrating system. The numbers shown in Table 1 for the average concentration factor and SDs were obtained by performing simple linear regression to the data. A *t*-test showed that the intercepts in the regression lines are all not significantly different from zero. Therefore, the average concentration factor can be expressed as the slope of the regression line, with the SD being the standard error of the slope.

The concentrations of fine mass and sulfates for a minor flow ratio of 0.2, shown in Figures 3 and 4, respectively, indicate that the fine mass concentration was increased by a factor of 26.3 (±2.7),

HMIs have been designed and characterized to have a 50% aerodynamic diameter cutpoint of 2.5 μm at a flow rate of 4 l/min (*15*). In addition, they operate with negligible interstage particle losses (<0.2%) for particles smaller than 2.7 μm in aerodynamic diameter. The experiment duration varied from 24 to 48 hr, depending on the observed air quality levels. Measurements of the concentrated aerosol were conducted by placing 47 mm Teflon filters downstream of the minor flow of stage III. Fine particulate mass and sulfate concentrations (d_p<2.5 μm) were determined by both sampling methods. The ratio of the concentration in the minor flow of stage III to the average concentration of the two HMIs gives an estimate of the ambient particle concentration levels that were achieved. The filters were preweighed on a Cahn 31 electrobalance in a constant temperature and humidity-controlled room. At the end of each run, the filters were weighed after equilibration. After weighing, we cut the filters and placed them in a polyethylene vial. The filters were extracted with 5 ml of ultrapure water and 0.100 ml ethanol, which wets the hydrophobic Teflon filters. Subsequently, the filters were sonicated for 15 min and analyzed for sulfate ions by ion chromatography (*16*).

Table 1. Summary of the results of the experimental characterization of the concentrating system

Minor flow ratio (stage III)		Fine mass		Sulfate	
		Range (μg/m^3)	Concentration factor[a] (SD)	Range (μg/m^3)	Concentration factor[a] (SD)
0.1	Ambient	9.3–31.4	25.8 (4.3)	1.8–7.3	23.3 (1.2)
	Concentrator	213.6–710.3		46.4–176.0	
0.2	Ambient	5.8–19.2	26.3 (2.7)	1.3–4.1	28.7 (3.4)
	Concentrator	167.2–533.1		31.5–121.5	

[a]The concentration factor is the average of the concentration factors in each of the experiments. It is based on seven experiments for $q_m/Q_T = 0.1$ and nine experiments for $q_m/Q_T = 0.2$.

whereas the fine sulfate concentration was increased by a factor of 28.7 (±3.4). The concentrator and ambient fine mass and sulfate concentrations were highly correlated ($R^2 = 0.93$ and 0.91, respectively).

Figures 5 and 6 show the fine mass and sulfate concentrations for a minor flow ratio of 0.1. The fine mass and sulfate concentration factors achieved at the lower minor flow ratio are 25.8 (±4.3) and 23.3 (±1.2), respectively, and the degree of correlation between the ambient and sulfates is also high ($R^2 = 0.87$ and 0.98, respectively). If the particle collection efficiency and losses in the third virtual impactor remained unaffected by the decrease in the minor flow, the observed concentrations at $q_m/Q_T = 0.1$ would have been approximately twice those observed at $q_m/Q_T = 0.2$ because concentrated particles at $q_m/Q_T = 0.1$ are contained in half the air volume. However, the results suggest that the concentration factors did not increase, but possibly decreased at a reduced minor flow ratio. This is mainly due to an increase in the internal particle losses, as well as a decrease in the virtual impactor's collection efficiency. Increased particle losses with decreasing minor flow ratio have been observed in previous investigations on the characteristics of virtual impactors (18,19). The increase in the losses has been attributed to the fact that a higher minor flow results in a higher local velocity around the tip of the collection nozzle, and consequently a stronger vacuum is applied to the particles as they exit the acceleration nozzle and approach the proximity of the collection probe. A stronger force pulls more particles into the collection probe, resulting in lower deposition around the tip of the collection nozzle.

The results from the particle loss tests are shown in Table 2. As the experiments indicate, particle losses through the chamber are small (10% or less). This is an expected result because ambient particles of the accumulation mode that were used as the test aerosol are not small enough to diffuse to the walls and not large enough to settle due to gravity within the 6 minutes of their residence in the chamber. Loss due to gravitational deposition, E_G, in a hori-

zontal tube are given by the equation (20):

$$E_G = \frac{\pi V_{TS} L d_t}{4Q} \quad (1)$$

where L is the length of the tube (cm), Q is the flow rate through the tube (cm^3/sec), V_{TS} is the particle-settling velocity (cm/sec), and d_t is the tube diameter (cm).

The diffusion loss, E_D, for an aerosol flowing through a tube is given by the formula (20):

$$E_D = 1 - \frac{C_e}{C_o} = 3.77\mu - 5.50\mu^{2/3} \quad (2)$$
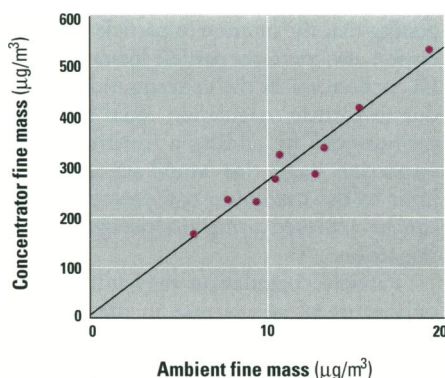
for $\mu < 0.007$

and

$$E_D = 1 - 0.819e^{-11.5\mu} + 0.0975e^{-70.1\mu} + 0.0325e^{-179\mu} \quad (3)$$

for $\mu > 0.007$

where C_e is the concentration of the aerosol at the exit of the tube, and C_o is the concentration of the aerosol at the entrance of the tube. The parameter μ is defined as:

$$\mu = \frac{DL}{Q} \quad (4)$$

where D is the particle diffusion coefficient (cm^2/sec), L is the length of the tube (cm),

Figure 3. Ambient and concentrator fine mass concentrations. The minor flow ratio of all three virtual impactors is 0.2 ($y = 5.4570 + 26.281x$, $R^2 = 0.931$).
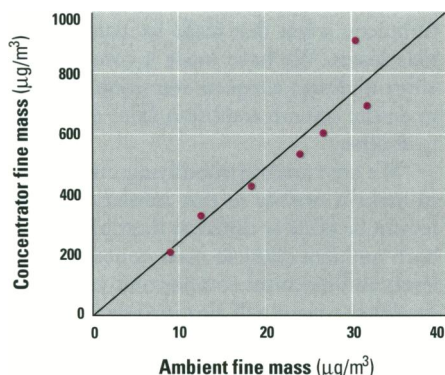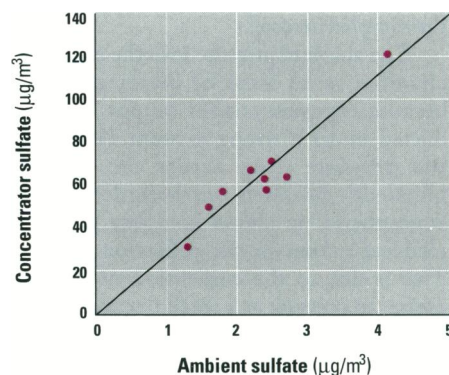
Figure 4. Ambient and concentrator sulfate concentrations. The minor flow ratio of all three virtual impactors is 0.2 ($y = -2.2915 + 28.749x$, $R^2 = 0.912$).

Figure 5. Ambient and concentrator fine mass concentrations. The minor flow ratio is 0.2 for stages I and II, and 0.1 for stage III ($y = -27.364 + 25.842x$, $R^2 = 0.877$).
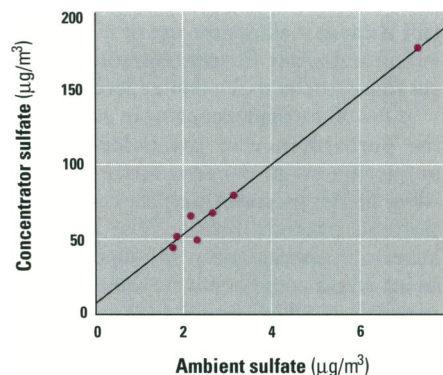
Figure 6. Ambient and concentrator sulfate concentrations. The minor flow ratio is 0.2 for stages I and II, and 0.1 for stage III ($y = 5.6861 + 23.308x$, $R^2 = 0.987$).

and Q is the flow rate through the tube (cm^3/sec). Table 3 shows the theoretically predicted particle losses due to gravitational settling and diffusion in the exposure chamber for particles in the size range 0.1–2.5 μm. Particle losses due to gravity become significant only for particles 2.5 μm in diameter, whereas losses due to diffusion are negligible for all particles. Other mechanisms, such as electrostatic deposition due to particle charge, may be responsible for the observed loss, especially since the exposure chamber is made of nonconductive material (plexiglass).

Discussion

The results presented here suggest that the use of several virtual impactor stages in series should be preferred over a single-stage virtual impactor operating at a low minor flow ratio to concentrate ambient particles. Higher minor flow ratios tend to increase the percentage of ambient fine particles that can be drawn through the collection probe of the impactor while decreasing the percentage of particles lost on the walls of the virtual impactor due to inertial and turbulent deposition. Hence, this configuration results in a minimum distortion of the initial size distribution of the sampled aerosol.

The absolute pressure into the exposure chamber is an issue of major concern because exposure studies cannot be conducted under a strong vacuum. Although the pressure drop across the virtual impactor's nozzle is on the order of 0.3 atmospheres, an almost complete pressure recovery occurs in the collection nozzle. This is due to the conversion of kinetic energy to pressure as a small fraction of the flow enters the collection nozzle, resulting in an absolute pressure of about 0.99 atmospheres (about 5 in H$_2$O vacuum). Because the concentrated aerosol is drawn through the collection nozzle, connecting several virtual impactor stages in series does not subject the concentrated particles to a substantial vacuum. This finding is important because it allows the use of multistage virtual impactors for concentrating ambient fine particles to the desired levels.

One of the main concerns in evaluating the performance of the three virtual impaction-stage concentrators was the effect of the increased particle concentration on particle losses. The gradual increase in concentration through the different stages could result in some particle coagulation, which would tend to increase particle deposition on the internal surfaces of the virtual impactors and transition pieces. The coagulation effect may become more pronounced in the lower stages, especially in the third stage, where the lower flow rates increase the particle residence

Table 2. Particle loss tests through the exposure chamber[a]

Experiment no.	Sulfate concentration (μg/m^3) Upstream	Sulfate concentration (μg/m^3) Downstream	Ratio upstream vs. downstream concentrations
1	57.5	54.2	0.94
2	42.2	38.8	0.92
3	24.2	22.0	0.91
4	56.1	50.3	0.90
5	31.5	30.5	0.97

[a]The supply flow rate is 10 l/min.

time in the various components of the system. The combined effect of the increase in the concentration and residence time may therefore decrease the overall concentration factor. The experimental results demonstrated that the overall concentration factor may be weakly affected by the increase in particle concentration. Since two virtual impaction stages increased the concentration by a factor of approximately 10, the placement of a third stage should have increased the concentration by a factor of approximately 32 (e.g., $10^{1.5}$). Nevertheless, the overall increase in the concentration, based on fine mass and particulate sulfate, was by a factor of 26.3 (±2.7) and 28.7 (±3.4), respectively, suggesting that the increase in particle concentration also increases particle losses in stage III. A decrease in the concentration factor due to particle coagulation could be more pronounced by adding a fourth virtual impaction stage; this would set an upper limit to the maximum concentration that can be achieved using multistage virtual impaction.

Particle coagulation may not be the only cause of an increase in the particle losses as we keep adding stages. It is not trivial to design appropriate transition pieces that connect the concentrated flow of one stage to the inlet of the next stage. The more stages are added, the more critical the design of these pieces becomes. The problem becomes even more complicated because the flow in the transition sections is turbulent, a fact that tends to increase particle losses. We have made a considerable effort to design adequate transition sections to ensure smooth transition from one stage to another.

We are currently modifying our system to make it mobile so that inhalation studies can be done at different locations where both ambient particle mass concentration levels and chemical composition vary. The modified system will also include temperature and relative humidity regulation equipment, a feature not included in the systems described in this work because all of the characterization experiments occurred indoors. Two straightforward methods can be used to regulate the rela-

Table 3. Theoretical calculations of particle losses in the exposure chamber due to gravitational settling and diffusion

Particle diameter (μm)	Gravitational loss (%)	Diffusional loss (%)
0.1	0.1	0.13
0.5	1.2	0.04
1.0	4.1	0.02
2.5	15.2	0.01

tive humidity and temperature of the concentrated aerosol before supplying it to the exposure chamber. First, the sampled air could be drawn through a relative humidity and temperature conditioning unit before passing through the series of virtual impactors. In this case, the air conditioner would have to be tested for particle losses to determine its suitability. Alternatively, the concentrated aerosol from stage III could be drawn through a fourth virtual impaction stage to further increase the concentration. Then the aerosol would be diluted with clean air before it is supplied to the chamber. The temperature and moisture content of the clean dilution air would be adjusted so that the resulting aerosol is supplied to the exposure chamber at the desired temperature and relative humidity. We are currently investigating both methods to determine which one is more practical.

Gas-phase pollutants such as ozone, ammonia, and sulfur dioxide can be removed from the concentrated aerosol before entering the exposure chamber by using glass honeycomb denuders, developed by Koutrakis et al. (13). These glass honeycomb denuders have high gas-removal efficiency and negligible internal particle losses. Removal of these gases allows the investigation of respiratory effects of particles alone. Moreover, it becomes possible to add gases back in a controlled manner to investigate interaction effects.

It should be noted that the present system does not increase the concentration of particles smaller than 0.1 μm. Although the mass fraction of these particles is small, recently, there has been increasing interest in their health effects because they are found in very high numbers in the atmosphere. The most important aspect of the fine ambient particle-concentrating method that we have developed is that it increases the concentration of particles in the size range 0.1–2.5 μm by a factor of 25–30 while maintaining their physicochemical characteristics and equilibria with gas-phase pollutants. In addition, it enables us to determine ambient particle toxicity by performing dose–response assessments of acute, subacute, and chronic exposures to real-time ambient aerosols.

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